1: Int Arch Allergy Appl Immunol 1990;91(2):118-23

Related Articles, Books, Protein, Nucleotide

Isolation of cDNA coding for the major mite allergen Der p II by IgE plaque immunoassay.

Chua KY, Doyle CR, Simpson RJ, Turner KJ, Stewart GA, Thomas WR.

Clinical Immunology Research Unit, Princess Margaret Hospital for Children, Subiaco, Australia.

A lambda gt11 library made with cDNA from the house dust mite Dermatophagoides pteronyssinus was screened with human allergic serum by IgE plaque radioimmunoassay. This resulted in the isolation of clones coding for the major allergen Der p II. The cDNA coded for a 129-residue protein of 14,131 daltons with no N-glycosylation sites. No sequence homology with other proteins was evident. The Der p II expressed in Escherichia coli reacted with IgE in 14 of 17 sera from mite-allergic patients giving clonal evidence for its designation as a major allergen. This, along with previous work, has resulted in the cloning of the two major mite allergens.

PMID: 2341191 [PubMed - indexed for MEDLINE]

1: A60381 **allergen Der p II precursor - house-dust mite (Dermatophagoides pteronyssinus)** BLink, PubMed, Related Sequences, Taxonomy

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LOCUS       A60381        146 aa                    INV       13-SEP-1998
DEFINITION  allergen Der p II precursor - house-dust mite  (Dermatophagoides
            pteronyssinus).
ACCESSION   A60381
PID         g280576
VERSION     A60381  GI:280576
DBSOURCE    pir: locus A60381;
            summary: #length 146 #molecular-weight 15999 #checksum 25;
            superfamily: allergen Der p II;
            PIR dates: 03-Mar-1993 #sequence_revision 03-Mar-1993 #text_change
            13-Sep-1998.
KEYWORDS    .
SOURCE      Dermatophagoides pteronyssinus.
  ORGANISM  Dermatophagoides pteronyssinus
            Eukaryota; Metazoa; Arthropoda; Chelicerata; Arachnida; Acari;
            Acariformes; Sarcoptiformes; Astigmata; Analgoidea; Pyroglyphidae;
            Dermatophagoides.
REFERENCE   1  (residues 1 to 146)
  AUTHORS   Chua,K.Y., Doyle,C.R., Simpson,R.J., Turner,K.J., Stewart,G.A. and
            Thomas,W.R.
  TITLE     Isolation of cDNA coding for the major mite allergen Der p II by
            IgE plaque immunoassay
  JOURNAL   Int. Arch. Allergy Appl. Immunol. 91 (2), 118-123 (1990)
  MEDLINE   90256301
FEATURES             Location/Qualifiers
     source          1..146
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     Protein         1..146
                     /product="allergen Der p II precursor"
     Region          18..146
                     /region_name="product"
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ORIGIN
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       61 nqntktakie ikasidglev dvpgidpnac hymkcplvkg qqydikytwn vpkiapksen
      121 vvvtvkvmgd dgvlacaiat hakird
//
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Limited proteolysis of tetanus toxin. Relation to activity and identification of cleavage sites.

Krieglstein KG, Henschen AH, Weller U, Habermann E.

Department of Molecular Biology and Biochemistry, University of California, Irvine 92717.

Tetanus toxin is synthesized by Clostridium tetani as a 151-kDa peptide chain. The primary gene product is processed post-translationally by removal of the initiating methionine residue, formation of disulfide bridges and limited proteolysis by bacterial or exogenous proteinases. The mature toxins consist of a 52-kDa light chain and a 98-kDa heavy chain, linked together by a disulfide bond. Proteolytic nicking is accompanied by increased pharmacological potency. To identify the structural alterations involved, single-chain toxin has been subjected to limited proteolysis with various enzymes. The new N-termini have been determined by Edman degradation and the C-termini by isolation of short C-terminal peptide fragments and subsequent analysis of the sequence and composition. All two-chain toxins result from proteolytic nicking within the 17-residue segment of residues 445-461. Thus, the protease(s) of the culture broth cleave on the C-terminal side of Glu449 and partially Ala456, giving rise to two heavy chain N-termini. Trypsin and clostripain first attack the C-terminal of Arg454 and later Arg448, whereas endoproteinase Arg-C cleaves the former bond only. Chymotrypsin and endoproteinase Glu-C each split a single peptide bond, i.e. that located after Tyr452 and Glu449, respectively. Papain gives rise to a large number of cleavages within the 17-residue segment, the new C-terminus being Thr445 or Asn446 and the new N-terminus being Asp460 or Leu461. Further papain digestion leads to an additional cleavage within the heavy chain between Ser863 and Lys864. The original N-terminal Pro1 and C-terminal Asp1314, predicted from the nucleotide sequence, are conserved in all proteolytic digests. The pharmacological activity of the various two-chain toxins was 5-11 times that of the single-chain toxin, as estimated from the inhibition of [3H]noradrenaline release from rat-brain homogenate. The present data on the processing and activation by limited proteolysis prove the existence of several active tetanus isotoxins. These data, together with our previous data on the localization of disulfide bridges and sulfhydryl groups (Krieglstein, K., Henschen, A., Weller, U. & Habermann, E. (1990) Eur. J. Biochem. 188, 39-45), provide the detailed protein chemical

characterization of the tetanus isotoxins.

PMID: 1935979 [PubMed - indexed for MEDLINE]

1: P04958 **TETANUS TOXIN PRECURSOR (TENTOXYLYSIN)** BLink, PubMed, Related Sequences, Taxonomy

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LOCUS       TETX_CLOTE   1315 aa                    BCT       15-JUL-1999
DEFINITION  TETANUS TOXIN PRECURSOR (TENTOXYLYSIN).
ACCESSION   P04958
PID         g135624
VERSION     P04958  GI:135624
DBSOURCE    swissprot: locus TETX_CLOTE, accession P04958;
            class: standard.
            plasmid:,created: Aug 13, 1987.
            sequence updated: Aug 13, 1987.
            annotation updated: Jul 15, 1999.
            xrefs: gi: gi: 40769, gi: gi: 40770, gi: gi: 144920, gi: gi:
            144921, gi: gi: 40773, gi: gi: 40774, gi: gi: 69647, gi: gi:
            3212428, gi: gi: 3891994
            xrefs (non-sequence databases): PROSITE PS00142
KEYWORDS    Neurotoxin; Transmembrane; Hydrolase; Metalloprotease; Zinc;
            Plasmid; 3D-structure.
SOURCE      Clostridium tetani.
  ORGANISM  Clostridium tetani
            Bacteria; Firmicutes; Bacillus/Clostridium group; Clostridiaceae;
            Clostridium.
REFERENCE   1  (residues 1 to 1315)
  AUTHORS   Eisel,U., Jarausch,W., Goretzki,K., Henschen,A., Engels,J.,
            Weller,U., Hudel,M., Habermann,E. and Niemann,H.
  TITLE     Tetanus toxin: primary structure, expression in E. coli, and
            homology with botulinum toxins
  JOURNAL   EMBO J. 5 (10), 2495-2502 (1986)
  MEDLINE   87053814
  REMARK    SEQUENCE FROM N.A.
REFERENCE   2  (residues 1 to 1315)
  AUTHORS   Fairweather,N.F. and Lyness,V.A.
  TITLE     The complete nucleotide sequence of tetanus toxin
  JOURNAL   Nucleic Acids Res. 14 (19), 7809-7812 (1986)
  MEDLINE   87040747
  REMARK    SEQUENCE FROM N.A.
            STRAIN=CN3911
REFERENCE   3  (residues 1 to 1315)
  AUTHORS   Fairweather,N.F., Lyness,V.A., Pickard,D.J., Allen,G. and
            Thomson,R.O.
  TITLE     Cloning, nucleotide sequencing, and expression of tetanus toxin
            fragment C in Escherichia coli
  JOURNAL   J. Bacteriol. 165 (1), 21-27 (1986)
  MEDLINE   86085672
  REMARK    SEQUENCE OF 742-1314 FROM N.A.
REFERENCE   4  (residues 1 to 1315)
  AUTHORS   Krieglstein,K., Henschen,A., Weller,U. and Habermann,E.
  TITLE     Arrangement of disulfide bridges and positions of sulfhydryl groups
            in tetanus toxin
  JOURNAL   Eur. J. Biochem. 188 (1), 39-45 (1990)
  MEDLINE   90201034
  REMARK    PARTIAL SEQUENCE, AND DISULFIDE BONDS.
REFERENCE   5  (residues 1 to 1315)
```

AUTHORS Krieglstein,K.G., Henschen,A.H., Weller,U. and Habermann,E.
TITLE Limited proteolysis of tetanus toxin. Relation to activity and identification of cleavage sites
JOURNAL Eur. J. Biochem. 202 (1), 41-51 (1991)
MEDLINE 92037649
REMARK PARTIAL SEQUENCE.
REFERENCE 6 (residues 1 to 1315)
AUTHORS Schiavo,G., Poulain,B., Rossetto,O., Benfenati,F., Tauc,L. and Montecucco,C.
TITLE Tetanus toxin is a zinc protein and its inhibition of neurotransmitter release and protease activity depend on zinc
JOURNAL EMBO J. 11 (10), 3577-3583 (1992)
MEDLINE 93010948
REMARK IDENTIFICATION AS ZINC-PROTEASE.
REFERENCE 7 (residues 1 to 1315)
AUTHORS Schiavo,G., Benfenati,F., Poulain,B., Rossetto,O., Polverino de Laureto,P., DasGupta,B.R. and Montecucco,C.
TITLE Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin
JOURNAL Nature 359 (6398), 832-835 (1992)
MEDLINE 93063293
REMARK IDENTIFICATION OF SUBSTRATE.
REFERENCE 8 (residues 1 to 1315)
AUTHORS Umland,T.C., Wingert,L.M., Swaminathan,S., Furey,W.F., Schmidt,J.J. and Sax,M.
TITLE Structure of the receptor binding fragment HC of tetanus neurotoxin
JOURNAL Nat. Struct. Biol. 4 (10), 788-792 (1997)
MEDLINE 97475217
REMARK X-RAY CRYSTALLOGRAPHY (2.7 ANGSTROMS) OF 874-1314.
COMMENT ----------

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[FUNCTION] TETANUS TOXIN ACTS BY INHIBITING NEUROTRANSMITTER RELEASE. IT BINDS TO PERIPHERAL NEURONAL SYNAPSES, IS INTERNALIZED AND MOVES BY RETROGRADE TRANSPORT UP THE AXON INTO THE SPINAL CORD WHERE IT CAN MOVE BETWEEN POSTSYNAPTIC AND PRESYNAPTIC NEURONS. IT INHIBITS NEUROTRANSMITTER RELEASE BY ACTING AS A ZINC ENDOPEPTIDASE THAT CATALYZES THE HYDROLYSIS OF THE 76-GLN-|-PHE-77 BOND OF SYNAPTOBREVIN-2.
[CATALYTIC ACTIVITY] HYDROLYSIS OF 76-GLN-|-PHE-77 BOND IN SYNAPTOBREVIN.
[SUBUNIT] THE PRECURSOR POLYPEPTIDE IS SUBSEQUENTLY CLEAVED TO YIELD SUBCHAINS L AND H. THESE REMAIN LINKED BY A DISULFIDE BRIDGE AND ARE NON-TOXIC AFTER SEPARATION.
[MISCELLANEOUS] THE C-TERMINAL OF THE HEAVY CHAIN BINDS TO GANGLIOSIDE RECEPTORS.
[SIMILARITY] BELONGS TO PEPTIDASE FAMILY M27 (ZINC METALLOPROTEASE); ALSO KNOWN AS THE TETANUS/BOTULINUM NEUROTOXIN SUBFAMILY.

FEATURES	Location/Qualifiers
source	1..1315
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Protein	1..1315
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	/note="TETANUS TOXIN LIGHT CHAIN."
Region	227..247

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     Site            234
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     Site            237
                     /site_type="metal-binding"
                     /note="ZINC (CATALYTIC) (BY SIMILARITY)."
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                     /note="INTERCHAIN."
     Region          458..1315
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                     /note="TETANUS TOXIN HEAVY CHAIN."
     Region          670..690
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                     /note="POTENTIAL."
     Bond            bond(1077,1093)
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      121 syslldkfdt nsnsvsfnll eqdpsgattk samltnliif gpgpvlnkne vrgivlrvdn
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      241 glygmqvssh eiipskqeiy mqhtypisae elftfggqda nlisidiknd lyektlndyk
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      361 ielgkkfnik trlsyfsmnh dpvkipnlld dtiyndtegf nieskdlkse ykgqnmrvnt
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      481 knsfseepfq deivsyntkn kplnfnysld kiivdynlqs kitlpndrtt pvtkgipyap
      541 eyksnaasti eihniddnti yqylyaqksp ttlqritmtn svddalinst kiysyfpsvi
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      841 itelkklesk inkvfstpip fsysknldcw vdneedidvi lkkstilnld inndiisdis
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     1021 dkfnaylank wvfititndr lssanlying vlmgsaeitg lgairednni tlkldrcnnn
     1081 nqyvsidkfr ifckalnpke ieklytsyls itflrdfwgn plrydteyyl ipvassskdv
     1141 qlknitdymy ltnapsytng klniyyrrly nglkfiikry tpnneidsfv ksgdfiklyv
     1201 synnnehivg ypkdgnafnn ldrilrvgyn apgiplykkm eavklrdlkt ysvqlklydd
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Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (Dermatophagoides spp).

Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TA.

Department of Internal Medicine, University of Virginia Medical Center, Charlottesville 22908.

Monoclonal antibody affinity chromatography was used to purify two homologous mite allergens, Der f II from Dermatophagoides farinae and Der p II from D. pteronyssinus. They have the same molecular weight (MW) (15 kd) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, they have similar amino acid compositions, and their N-terminal amino acid sequences differ in only four of the first 35 residues. An excellent correlation was observed between IgE antibody to Der f II and Der p II measured in sera from 65 mite-allergic patients ($r = 0.94$; p less than 0.001) and between quantitative intradermal skin tests to both allergens. A third allergen (Der f III, MW 29 kd) was purified from D. farinae by repeated gel filtration. In sera from 51 mite-allergic patients, IgE antibody to Der f II, Der f III, and previously purified Der f I (MW 24 kd) was detected in 92%, 16%, and 78% of the sera by radioimmunoassay, respectively. Most patients, 41/51 (80%), demonstrated IgE antibody to more than one allergen. With monoclonal antibodies fully cross-reactive with Der f II and Der p II, a two-site immunoassay was developed for measuring absolute quantities (nanograms or micrograms) of these allergens. In extracts rich in mite-fecal material ($n = 5$), Der f I and Der p I (group I allergens) and Der f II and Der p II (group II allergens) were measured in ratios of 11:1 to 35:1. Lower ratios (1.1:1 to 7:1) were observed in mite body extracts ($n = 6$). These experiments clearly define a second group of major dust mite allergens that demonstrate extensive structural and antigenic homology.

PMID: 2732406 [PubMed - indexed for MEDLINE]